The thus obtained product was electrophoresed using 20% polyacrylamide 7M urea gel and analyzed by using imaging plate (Phosphoroimager analysis). Result is shown in Fig. 6. In Fig. 6, left five, i.e. AG, AGC, AGT, AGY and AGCT, indicates cases using template 1, and right five indicates cases using template 3. Results indicate that a base y was incorporated into complementary strands of A, G and X. To the complementary strand X was incorporated also C and T in addition to Y (refer to Fig. 6).

For quantitative analysis of the incorporation, same experiments were conducted by adding only dNTP (150 μ M) using primer 2 (1 μ M) labeled with p32 at 5'-terminal and template 1, 2 and 3 (2 μ M).

Primer and template used in the experiments are shown below.

A case using template 1:

A case using template 2:

A case using template 3:

Results are shown in Fig. 7 A and B. As a result, Y was incorporated into complementary strands of A, G and X at 78%, 48% and 41%, respectively, and Y, C and T were incorporated into complementary strand of X, at 41%, 9.5% and 13%, respectively. (Refer to Fig. 7)

Since Y was incorporated independently into not only X but also A and G, the following experiments were conducted in order to find out to what strands Y was incorporated when T and C were coexisted.

Primer 2 without labeling and template 2 were annealed, and [α-^{32}P] TTP and various amounts of dYTP were added thereto to find out ratio of inhibition of incorporation of [α-^{32}P] TTP into X by dYTP was investigated. Simultaneously with addition of dATP, effect of the said inhibition on incorporation of A into complementary strand of T next to X was investigated (refer to Fig. 8 A). As a result, when dYTP was added almost equivalent level of [α-^{32}P] TTP, incorporation of [α-^{32}P] TTP into X was inhibited at 50%. Same experiments were conducted by using template 1 and 2 for A and G. dYTP did not inhibit incorporation of [α-^{32}P] TTP into the complementary strand of A and the incorporation of [α-^{32}P] CTP into the complementary strand of G. (Refer to Fig. 8 B for template 1 and C for template 2). Consequently, incorporation of dYTP into A and G was suppressed by coexisting TTP and dCTP.

In order to search effect of incorporation of Y, C and T into the complementary strand of X, when two X are presented on the template, ^{32}P labeled primer 3 at 5'-terminal and template 4, 5, 6, 7, 8 and 9 were used for primer extension method. As a result, when two X were continued on the template, polymerase reaction was terminated at the position where two

functional expression of natural gene. Such the artificial nucleic acid base pair of the present invention can be applied not only on the protein synthesis system or functional nucleic acid but also on the solution of functions and elucidation of natural gene systems.

Examples

Following examples illustrate the present invention but are not construed as limiting the present invention.

Example 1:

Synthesis of 2-benzamino-6-(N,N-dimethylamino)-9-[5'-O-dimethoxytrityl-3'-O-[[(diisopropylamino)-2-cyanoethoxy] phosphino]-2'-deoxy-β-D-ribofuranosyl] purine (10) (refer to Fig. 3)

(A) Synthesis of 2-amino-6-(N,N-dimethylamino)-9-(2',3,5'-tri-O-acetyl-β-D-ribofuranosyl] purine (2):

2-amino-6-chloro-9-(2',3',5'-tri-O-acetyl-β-D-ribofuranosyl) purine (1) [M. J. Robins and B. Uznanski, Can. J. Chem., 59, 2601-2607 (1981)](18.6 mmol, 7.96 g) was dehydrated three times azeotropically with anhydrous pyridine, and dissolved in anhydrous pyridine (180 ml), then dimethylamine hydrochloride (55.8 mmol, 4.55 g) and diisopropylethylamine (74.4 mmol, 12.9ml) were added thereto with stirring at room temperature. The mixture was stirred at room temperature for 15 hours. After confirming completion of the reaction by TLC, water was added to the reaction mixture and

5.5 Hz), 4.07-4.20 (m, 2H, H4', H5'), 4.06 (d, 1H, H5", J = 13.0 Hz), 3.48 (br, 6H, N-CH_3), 0.95-1.08 (m, 28H, iPr).

(E) Synthesis of 2-benzamino-6-(N,N-dimethylamino)-9-(2'-O-phenoxythiocarbonyl-3',5'-O-tetraisopropyldisiloxanyl-β-D-ribofuranosyl) purine (6)

The compound (5) obtained in the above (D) (3.98 mmol, 2.61 g) was dehydrated azeotropically three times with anhydrous toluene and dissolved in anhydrous dichloromethane (40 ml). 1-methyimidazole (7.96 mmol, 0.64 ml) and chlorothio carbonate phenyl (5.57 mmol, 0.77 ml) were added with stirring at room temperature, then stirred at room temperature for 16 hours. After confirming completion of the reaction by TLC, 5% aqueous sodium hydrogen carbonate was added to the reaction mixture. After extracted the organic layer, the organic layer was washed one with aqueous 5% sodium hydrogen carbonate, once with water, twice with aqueous 10% citrate solution and once with water, in this order, the organic layer was dried with magnesium sulfate, filtered and dried in vacuo. The residue was purified by silica-gel column chromatography (dichloromethane-methanol) to obtain the product (6) 2.96 g (3.73 mmol) (94%).

^{1}H-NMR (500.13 MHz, DMSO-d_6) δ : 8.17 (s,1H, H8), 7.87 (d, 2H, Bz-m, J = 3.0 Hz), 7.79 (s, 1H, NHBz), 7.55 (t, 1H, Bz-p, J = 7.5 Hz), 7.47 (t, 2H, H Bz-o, J = 7.5 Hz), 7.41 (d, 2H, PhO-o, J = 7.5 Hz), 7.29 (t, 2H, PhO-m, J = 7.5 Hz), 7.13 (d, 1H, PhO-p, J = 10.0 Hz), 6.39 (d, 1H, H2', J = 5.0 Hz), 6.11 (s, 1H, H1'), 5.14-5.17 (m, 1H, H3'), 4.23-4.26 (m, 1H, H5'), 4.07-4.12 (m, 1H, H4', H5"), 3.48 (br, 6H, N-CH_3), 0.99-1.15 (m, 28H, iPr).

(F) Synthesis of 2-benzamino-6-(N,N-dimethylamino)-9-(2'-deoxy-3',5'-O-tetraisopropyldisiloxanyl-β-D-ribofuranosyl) purine (7)

The compound (6) obtained in the above (E) (3.73 mmol, 2.96 g) was dehydrated azeotropically three times with anhydrous toluene, and dissolved in anhydrous toluene (88 ml). 2,2'-azo-bis-isobutyronitrile (0.746 mmol, 122 mg) was added thereto with stirring at room temperature and added argon gas with bubbling for 1 hours at room temperature. Thereto was added tributyltin hydride (5.60 mmol, 1.51 ml) and stirred at 75℃ for 3.5 hours. After confirming completion of the reaction by TLC, the reaction mixture was concentrated in vacuo. The residue was purified by silica-gel column chromatography (dichloromethane-methanol) to obtain the product (7) 2.27 g (3.55 mmol) (95%).

^{1}H-NMR (500.13 MHz, $CDCl_3$) δ : 8.24 (s, 1H, H8), 7.90 (d, 2H, Bz-m, J = 5.0 Hz), 7.83 (s, 1H, NHBz), 7.54 (t, 1H, Bz-p, J = 7.5 Hz), 7.48 (t, 2H, H Bz-o, J = 7.5 Hz), 6.29 (dd, 1H, H1', J = 7.5 Hz), 4.80-4.83 (m, 1H, H3'), 3.97-4.07 (m, 2H, H5', H5"), 3.86-3.88 (m, 1H, H4'), 3.50 (br, 6H, N-CH_3), 2.68-2.71 (m, 1H, H2'), 2.59-2.63 (m, 1H, H2"), 1.03-1.09 (m, 28H, iPr).

(G) Synthesis of 2-benzamino-6-(N,N-dimethylamino)-9-(2'-deoxy-β-D-ribofuranosyl) purine (8)

The compound (7) obtained in the above (F) (3.55 mmol, 2.27 g) was added to 1M solution of tetrabutylammonium fluoride - tetrahydrofuran (14 ml) and stirred at room temperature for 15 minutes. After confirming

ribofuranosyl) purine (21)

The compound (20) 98 mg (0.24 mmol) obtained in the above (C) was azeotropically distilled three times with anhydrous pyridine 1 ml. The residue was dissolved in anhydrous pyridine 2 ml, added triethylamine 35 ml, dimethylaminopyridine 1.4 mg and dimethoxytrityl chloride 85 mg were added thereto and stirred at room temperature for overnight. Ethyl acetate 25 ml was added to the reaction mixture. The mixture was treated with water 25 ml for three time for separation to obtain organic layer. Each aqueous layer was washed with ethyl acetate. The organic layer was collected, dried with anhydrous sodium sulfate and concentrated in vacuo. The residue was purified by using short column (developer: 25 - 50% ethyl acetate - dichloromethane) to obtain the product (21) 132 mg (0.19 mmol) (76.7%).

^{1}H-NMR (270 MHz, $CDCl_3$) δ :

8.64 (dd, J = 3.6, 0.9 Hz, 1H), 8.14 (s, 1H), 7.92 (bs, 1H), 7.61 (dd, J = 4.3, 0.9 Hz, 1H), 7.39 (m,2H), 7.24 (m, 8H), 6.77 (m, 4H), 6.47 (t, J = 6.2 Hz, 1H), 4.79 (m, 1H), 4.13 (m, 1H), 3.76 (s, 3H), 3.75 (s, 3H), 3.44 (dd, J = 10.23, 5.8 Hz, 1H), 3.38 (dd, J = 10.23, 4.4 Hz, 1H), 2.91 (m, 1H), 2.60 (m, 1H), 2.30 (m, 1H), 1.27 (m, 6H).

(E) Synthesis of 2-isobutyrylamino-6-(2-thienyl)-9-[2-deoxy-3-O-[(diisopropylamino)-(2-cyanoethoxy)] phosphyno-5-O-dimethoxytrityl-β-D-ribofuranosyl) purine (22)

The compound (21) 125 mg (0.18 mmol) obtained in the above (D) was azeotropically distilled three times with anhydrous pyridine 0.5 ml and azeotropically distilled three times with anhydrous tetrahydrofuran 0.5 ml.

1H, H1'), 4.37-4.40 (m, 1H, H3'), 3.97-4.12 (m, 2H, H5', H5"), 3.80-3.84 (m, 1H, H4'), 2.26-2.36 (m, 1H, H2'), 1.77-1.86 (m, 1H, H2"), 0.90-1.09 (m, 28H, iPr).

(D) Synthesis of 3-(2'-deoxy-β-D-ribofuranosyl) pyridine-2-one (15)

The compound (14) (0.089 mmol, 42 mg) obtained in the above (C) was dehydrated azeotropically three times with anhydrous toluene, added 1 M tetramethyl ammoniumfluoride/THF solution (0.5 ml) and stirred at room temperature for 2 hours. After confirming completion of the reaction by TLC, acetic acid (0.08 ml) was added thereto and concentrated in vacuo. The residue was dissolved in water, washed three times with ethyl acetate, and the aqueous layer was concentrated in vacuo. The residue was purified by using reverse phase silica-gel chromatography to obtain the product (15) 10.4 mg (0.047 mmol) (52%).

^{1}H-NMR (270.06 MHz, $CDCl_3$) δ : 7.77 (d, 1H, H4, J = 3.8 Hz), 7.36 (d, 1H, H6, J = 3.5 Hz), 6.41 (t, 1H, H5, J = 3.6 Hz), 5.01-5.17 (m, 1H, H1'), 4.29-4.31 (m, 1H, H3'), 3.93-3.95 (m, 1H, H4'), 3.62-3.70 (m, 2H, H5', H5"), 2.31-2.35 (m, 1H, H2'), 1.89-1.95 (m, 1H, H2").

(E) Synthesis of 3-(2'-deoxy-5'-O-triphosphoryl-β-D-ribofuranosyl) pyridine-2-one (16)

The compound (15) (0.059 mmol, 13.4 mg) obtained in the above (D) was dehydrated azeotropically three times with anhydrous toluene, dissolved in trimethyl phosphate (0.2 ml), added phosphorus oxychloride (0.065 mmol, 7.1 μl) under ice-cooling and stirred for 7 hours under ice-cooling. After confirming completion of the reaction by TLC, well mixed solution of 0.5 M

3 hours. After the reaction, 10 M urea dye was added and kept at 75℃ for 3 minutes, then electrophoresed with 20% polyacrylamide gel. The product was analyzed. Result is shown in Fig. 10.

Example 9:

Transcription using T7 RNA polymerase

Reaction was performed as same as in example 8. The generated RNA was isolated by gel electrophoresis. RNA was digested by 0.75 units RNase T2. Each nucleotide was separated using 2-dimension TLC and each ratio was calculated.

Result is shown in Fig. 11. Ratio of composition of each nucleotide is shown in Table 1 hereinbefore.

Example 10:

Single nucleotide insertion reaction using Klenow fragment (exo^+)

A solution containing [5'-^{32}P] labeled primer DNA (20-mer, 4 mM), template DNA (35-mer, 4 mM) and 2x Klenow fragment buffer (TAKARA) were annealed at 95℃ for 3 minutes, 40℃ for 3 minutes and 4℃ for 7 minutes. A solution of equimolar amount of 40 mM dNTP and Klenow fragment (exo^+) (2 unit/ml, For Sequencing, TAKARA) were added thereto and incubated at 37℃ for 30 minutes. Equimolar amount of 10 M urea BPB dye solution was added and kept at 75℃ for 5 minutes and electrophoresed with 20% polyacrylamide - 7M urea gel. Products were analyzed by using Phosphoroimager plate. Result is shown in Fig. 12.

Example 11:

Transcription by T7 RNA polymerase

A solution containing template DNA 1mM, in which promoter region has duplicated strands, T7 RNA polymerase 2.5 units, 2mM rNTP, and [α -^{32}P] rATP 0.1mCi/ml [40 mM Tris-HCl (pH 8.0), 8 mM $MgCl_2$, 2 mM spermidine, 5 mM DTT, 0.01% Triton X-100, 10 mM rGMP] were prepared and incubated for 3 hours. 10 M urea dye was added and kept at 75℃ for 3 minutes to terminate the reaction. The product RNA (16-mer) in this solution was purified by using electrophoresis with 20% polyacrylamide gel. RNA was digested by 0.75 units RNase T2. Ratio of each nucleotide was determined by 2-dimenstion TLC (cellulose resin). In Fig. 13, result of development of TLC is shown. Ratio of each nucleotide is shown in Table 2 hereinbefore.

Example 12:

Synthesis of primer and template containing base X2

Primer and template were synthesized conventionally by using DNA/RNA synthesizer Type 392, The Perkin-Elmer, Applied Biosystems Div., and cyanoethylamidide reagents of dA, dC, dG and dT, which were distributed by The Perkin-Elmer, and dx2 of cyanoethylamidite reagent prepared according to the method in example 1.

Proviso that in a synthesis of oligomer containing dx2, removal of protective group for 2-amino group of dx2, i.e. isobutyryl group, could not completely be performed, under the usual basic condition after synthesis of oligomer (conc. ammonia at 55℃ for 10 hours), consequently, treatment for removal of the protective group was performed under the condition at 80℃

group to be able to form additional hydrogen bonds.

10. The group to be able to form additional hydrogen bond is an electron pair of amino group, hydroxyl group, keto group or nitrogen group.

11. The method according to any of claims 1 - 10 wherein the base pair is a base pair which can be recognized by polymerase.

12. The method according to claim 11 wherein the polymerase is DNA polymerase or RNA polymerase.

13. A method for designing nucleic acid to construct selective base pair comprising utilizing steric hindrance in the nucleic acid base part.

14. A method for designing nucleic acid to construct selective base pair comprising hindering to construct base pair with the natural nucleic acid base part by utilizing the steric hindrance.

15. A method for designing nucleic acid to construct selective base pair comprising utilizing steric hindrance and electrostatic repulsion, and stacking action in the nucleic acid base part.

16. A method for designing nucleic acid to construct selective base pair comprising hindering to construct base pair with the natural nucleic acid base part by utilizing steric hindrance and electrostatic repulsion and stabilizing to impart stacking action.

17. The method for designing nucleic acid according to any of claims 13 - 16 wherein the nucleic acid base pair is a base pair which can be recognized by polymerase.

18. A nucleic acid comprising being designed by the method according to any of claims 13 - 17.

19. The nucleic acid according to claim 18 wherein the nucleic acid has

be produced by the process according to claim 35 or claim 36.

40. A method for screening functions of amino acids coded by natural gene comprising using the non-natural gene which can be produced by the method according to claim 35 or claim 36.